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Re: Written comments on the NTP-CERHR Draft Expert Panel Report on Soy Formula (FR Doc. E9–25122

Dear Dr. Thayer:

The following comments are submitted in response to the October 19, 2009, draft NTP-CERHR Expert Panel Report on Soy Formula. I, Dr. Hans H. Stein, Ph.D., am an associate professor of animal nutrition at the University of Illinois with expertise in feed ingredient evaluation and nutrient utilization in pigs and other animal species. I am also the interim executive director for the National Soybean Research Laboratory in Urbana, IL.

As a nutritionist, I want to draw your attention to the practices that are used to include soybean products in diets fed to swine. In the US alone, more than 100 million pigs are produced every year and more than 95% of these pigs are produced on diets containing soybean meal as the primary protein source. The levels of isoflavones are much greater in soybean meal than in the soybean proteins used in infant formulas, which results in pigs receiving much greater levels of soy isoflavones than human infants. Because the metabolism of soy isoflavones is similar in pigs and humans it is of interest to review the use of soybean products in diets fed to swine.

INTRODUCTION

Soybean products are included in most rations fed to swine in the US and in most countries in the world because soybean protein is recognized at the premier protein source for pigs (Shelton et al., 2001). Intact soybeans may be used in swine feeding Baker & Stein, 2009), but soybean meal that is produced after the removal of fat from intact or de-hulled soybeans is by far the most common protein source used for all categories of pigs (Cromwell, 2000). Processed products such as soy protein concentrate or soy protein isolate are sometimes used in diets fed to weanling pigs. Newer enzymatically treated or fermented soybean products such as Hamlet Protein or PepSoyGen have been recently introduced to the feed industry and are mainly used in diets fed to weanling pigs (Pahm & Stein, 2007). The majority of soybean products included in diets fed to pigs are used to increase the dietary concentrations of amino acids (**AA**). However, soybean oil may also be used in diets fed to weanling, growing, and finishing pigs as an important source of energy (Mahan, 1991; Owens et al., 1996). It has also been suggested that soybean hulls that are produced by de-hulling soybean seeds may be included in diets fed to sows and growing-finishing pigs in quantities of up to 15% (Kornegay, 1981). However, because of the high concentration of fiber in soybean hulls, the digestibility of energy and most dietary nutrients, including AA, is reduced if soybean hulls are included in the diets (Kornegay, 1981; Dilger et al., 2004). Therefore, soybean hulls are usually not used in diets fed to swine.

NUTRIENTS AND ENERGY IN SOYBEAN PRODUCTS

Nutrient and Energy Concentrations in Soybean Products

Although all soybean products except soybean oil are included in diets fed to swine to increase the concentration of AA in the diets, soybean products also contribute energy and other nutrients. Energy, AA, and phosphorus (**P**) are the most important components in soybean meal, but it should be recognized that soybean products supply significant quantities of vitamins and many minerals to swine diets.

The concentration of energy, AA, and P in full fat soybeans as well as soybean meal, soy protein concentrate, and soy protein isolate has been published (NRC, 1998; Table 1). The concentration of gross energy (GE) in soybean meal is relatively constant across sources of soybean meal collected at different locations (van Kempen et al., 2006). However, the processing procedure used to produce the soybean meal has an impact on the total amount of energy in the meal. Expelled meals usually contain more energy than solvent extracted meals because expelled meals have a greater concentration of fat (Woodworth et al., 2001; Baker & Stein, 2009). Likewise, de-hulled soybean meal contains more energy than non-dehulled soybean meal (Woodworth et al., 2001).

Compared with most other protein sources, soybean protein has a relatively high concentration of lysine and tryptophan. The concentration of these two AA is relatively low in most cereal grains, and in particular in corn. Therefore, lysine is the first limiting AA in grain-based diets fed to pigs, and tryptophan is the second limiting AA in diets based on corn (Sharda et al., 1976). The AA profile of soybean protein, however, complements the AA profile of cereal grains because of the relatively high concentrations of lysine and tryptophan.

Variability in the nutrient composition of different sources of soybeans exists. This is true not only if soybeans and soybean meals are obtained from different countries, but also for samples obtained from different locations within the US (Grieshop et al., 2003; Karr-Lilienthal et al., 2004). However, the average concentration of nutrients in 10 samples of soybeans and in 10 samples of soybean meal obtained from different locations in the US are in good agreement with the values published by NRC (Grieshop et al., 2003). Likewise, when samples of non-dehulled and de-hulled soybean meal from 16 different sources in the US were analyzed for AA, average values that are close to NRC values were obtained (Cromwell et al., 1999). There is, therefore, good agreement on the average nutrient composition in soybean products, but it is also recognized that variability among sources may exist. Within the US, concentrations of protein and AA in soybean products is reduced for soybeans grown in the northern part of the country as compared with the central or southern states (Cromwell et al, 1999; Grieshop et al., 2003). In addition, newer varieties of soybeans that have been specifically selected for greater concentrations of proteins are now available and soybean meals obtained from these beans have a greater concentration of protein and amino acids than meals produced from conventional soybeans (Cervantes-Pahm & Stein, 2008; Baker & Stein, 2009).

Amino Acid Digestibility in Soybean Proteins

The apparent and standardized ileal digestibility of AA in soybean meal (with or without hulls) and in other soybean products has been measured in numerous experiments and results have been summarized (NRC, 1998; Table 2). The digestibility of AA in soybean meal collected from different geographical locations in the US is relatively constant (van Kempen et al., 2002). All sources of soy protein need to be heated prior to feeding to inactivate anti-nutritional factors, mainly protease inhibitors, present in raw soybeans (Qin et al., 1996). However, the form of heat applied to soy protein may influence the digestibility of AA in the product (Woodworth et al., 2001; Opapeju et al., 2006) and several other factors have been shown to influence the digestibility of AA in soy protein. In general, the more processed the soy protein is, the greater is the digestibility of AA. Therefore, AA in soy protein isolate and soy protein concentrate are usually more digestible than AA in soybean meal (Cervantes-Pahm & Stein, 2008), and AA in de-hulled soybean meal have a greater digestibility than AA in non-dehulled soybean meal (NRC, 1998). The latter observation is consistent with reports showing that soybean hulls reduce the digestibility of AA in soybean meal (Dilger et al., 2004). It is also likely that the reason for the increased digestibility of AA in soyprotein isolate compared with soybean meal is that fiber and oligosaccharides are removed from the defatted meals, which may impact the digestibility of AA (Smirickey et al., 2002).

The digestibility of AA in full fat soybeans is greater than in soybean meal (Cervantes-Pahm & Stein, 2008; Baker & Stein, 2009). The reason for this observation is most likely that the addition of oil to soybean meal or soy protein concentrate increases the digestibility of AA (Li and Sauer, 1994; Albin et al., 2001). In fact, Cervantes-Pahm & Stein (2008) demonstrated that the addition of soybean oil to soybean meal resulted in an increase in the digestibility of AA in soybean meal to a level that was not different from the digestibility obtained in full fat soybeans. Based on these observations, it is concluded that the reason for the greater digestibility of AA in full fat soybeans compared with soybean meal is the presence of fat in full fat soybeans.

The particle size of soybean meal also influences the digestibility of AA and the digestibility is improved in soybean meal having a particle size of 600 microns compared with soybean meal having a particle size of 900 microns (Fastinger & Mahan, 2003). This observation concurs with results from other experiments showing that performance of pigs fed diets based on corn and soybean meal will improve if the particle size is reduced (Lawrence et al., 2003). Microbial phytase does not influence ileal digestibility of AA in soybean meal (Traylor et al., 2001), but measured values for the standardized ileal digestibility of AA will be reduced as feed intake is increased (Motor & Stein, 2004). This observation has mainly implications for pigs fed experimental diets used to measure AA digestibility of soybeans because under commercial conditions, most pigs are allowed ad libitum access to feed.

Phosphorus Digestibility in Soybean Products

Historically, values for relative availability of P rather than digestibility of P have been measured (Cromwell et al., 1993) and relative availability values of 31 and 23% for not-dehulled and de-hulled soybean meal, respectively, have been reported (NRC, 1998). However, values for the relative availability of P in other soybean products are not available.

The apparent total tract digestibility of P in de-hulled soybean meal has been reported at 38 to 50% (Bohlke et al., 2005; Almeida & Stein, 2009; Akinmusire & Adeola, 2009). Apparent total tract digestibility of P in non-dehulled soybean meal was reported at 48.1 and 34.9% (Ajakaiye et al., 2003). However, Rodehutscord et al. (1996)

measured a value of only 31% for apparent total tract digestibility of P in non-dehulled soybean meal. Based on these results it can, therefore, be concluded that the values for relative availability of P in soybean meal that are published by NRC probably are too low, because the apparent total tract digestibility of P in soybean meal seems to be between 30 and 50% for both de-hulled and non-dehulled meal.

The digestibility of P in soybean meal can be improved by more than 100% if microbial phytase is added to the diet (Rodehutscord et al. 1996; Cromwell et al., 1993; Traylor et al., 2001; Almeida & Stein, 2009). Dietary microbial phytase, therefore, is very effective in improving the digestibility of P in soybean meal.

Energy Digestibility in Soybean Products

The digestibility of energy has not been reported from many experiments, but values for digestible energy (**DE**) and metabolizable energy (**ME**) in de-hulled soybean meal of 3,685 and 3,380 kcal/kg are published by NRC (1998). These values are in good agreement with recently measured values (Woodworth et al. (2001; Baker & Stein, 2009), but greater than the average DE value of 3,383 kcal DE per kg calculated from van Kempen et al (2006). Soybean meal from beans that have not been dehulled contains less digestible energy than dehulled soybean meal (3,490 and 3,180 kcal/kg DE and ME, respectively), whereas soy protein concentrate and soy protein isolate contain slightly more DE and ME than dehulled soybean meal (NRC, 1998). However, full fat soybeans contain more energy (4,140 and 3,690 kcal DE and ME, respectively) than any of the defatted soybean products. This observation is in agreement with the fact that soybean oil has a high concentration of energy (8,750 and 8,400 kcal DE and ME, respectively), which is the reason soybean oil can be added to diets to increase the energy value of the diet.

UTILIZATION OF SOYEBAN PRODUCTS IN DIETS FED TO PIGS

Soybean Meal

Soybean meal is one of the best protein sources that are available for swine diets (Shelton et al., 2001) and both de-hulled and non-dehulled soybean meals are excellent sources of AA for swine. However, new varieties of soybeans are constantly being developed using traditional plant breeding. These new varieties have specific nutritional characteristics that influence the quality of the soybean meal being produced from the beans. Examples of such new varieties are soybeans with higher protein concentration or lower concentrations of oligosaccharides, but there is only limited information about the nutritional value of these varieties as compared with conventional varieties (Cervantes-Pahm & Stein, 2008; Baker & Stein, 2009). Most of the soybeans that are grown were developed using genetically modified seeds that have specific agronomic traits. However, the nutritional composition and the feeding value of soybean meal produced from these beans are not different from the nutritional value of conventional soybeans (Cromwell et al., 2002).

In diets fed to growing-finishing and reproducing swine, all AA needed by the animals may be provided by soybean meal. However, newly weaned pigs do not tolerate soy protein as well as older pigs (Sohn et al., 1994), and they may develop allergenic reactions followed by immunological responses if they are fed large quantities of soybean meal (Li et al., 1990; 1991). It is, therefore, recommended that the concentration of soybean meal is limited in diets fed to pigs immediately after weaning and other protein sources need to be used in these diets. However, as the pigs grow older, the inclusion rate of soybean meal can be gradually increased, and when the pig reach a weight of 20 to 25 kg, soybean meal can be used as the only protein source in the diet.

To prevent the allergenic reactions of including soybean meal in diets fed to newly weaned pigs, soy protein concentrate and soy protein isolate may be used in diets fed to weanling pigs in stead of soybean meal. These protein sources may be used as the sole source of AA in diets fed to weanling pigs because they are thought not to elicit antigenic responses in the pigs (Sohn et al., 1994). The cost of soy protein isolate and soy protein concentrate is usually greater than the cost of soybean meal and the usage of these two protein sources is, therefore, usually limited to the diets fed from weaning and until pigs reach a body weight of 20 to 25 kg.

Two new soybean products, HP 300 and PepSoyGen, respectively, are devoid of soy allergens and were recently introduced to the North American marked. Because of the removal of soy-antigens from these products, they can be included in diets fed to weanling pigs without causing adverse allergenic reactions.

During the production of HP 300 (Hamlet Protein, Horsens, Denmark), a proprietary enzymatic preparation is used to digest the antigens in soybean meal. The oligosaccharides and sugars in the soybean meal are also removed and the resultant soybean meal contains approximately 53% crude protein (Table 3; Zhu et al., 1998). The digestibility of amino acids in HP 300 is greater than in conventional soybean meal (Table 3; Cervantes-Pahm, 2008). Numerous experiments in Europe and Asia have demonstrated that inclusion of HP 300 in diets fed to weanling pigs results in pig performance that is similar to that obtained on diets based on animal proteins such as milk protein and fish protein.

PepSoyGen (NutraFerm, North Sioux City, SD) is produced by fermentation of soybean meal in the presence of *Apergillus oryzae* and *Bacillus subtillis*. Antigens, oligosaccharides, and sugars are removed from the soybean meal during fermentation (Table 3; Hong et al., 2004; Yang et al., 2007). The proteins in the soybean meal are also hydrolyzed during fermentation, which results in reduced peptide size in PepSoyGen compared with conventional soybean meal (Hong et al., 2004). PepSoyGen contains approximately 10% more protein than conventional soybean meal, but the amino acid sequence is similar to the sequence in conventional soybean meal (Hong et al., 2004). The standardized ileal digestibility of amino acids in PepSoyGen is similar to the digestibility in conventional soybean meal (Table 4; Pahm and Stein, 20007), but the inclusion of PepSoyGen in diets fed to weanling pigs at the expense of conventional soybean meal improves pig performance (Feng et al., 2007). PepSoyGen can, therefore, be used in weanling pig diets as a substitute for more expensive animal protein sources.

Soybean Oil

Soybean oil is recognized as an excellent energy source in diets fed to all categories of swine. Addition of fat to diets fed to weanling pigs during the initial two weeks post-weaning, usually does not increase performance. However, from approximately day-15 post-weaning and during the remaining nursery period, average daily gain may be improved if soybean oil is added to the diet (Howard et al., 1990; Owen et al., 1996) although that is not always the case (Hoffman et al., 1993; Tokach et al., 1995). Diets containing soybean oil usually have a greater energy concentration than diets containing no soybean oil, and feed utilization is, therefore, often improved if measured on a kg per kg basis (Owen et al,. 1996). However, if measured on the basis of calories used per unit of gain, there is no effect of adding soybean oil to the diet (Hoffman et al., 1993).

In diets fed to growing pigs, fat addition often improves daily gain, but that is not always the case for finishing pigs (Overland et al., 1999; de la Llata et al., 2001). Feed utilization is usually not improved if measured on a calorie basis. Soybean oil is an excellent source of fat for growing and finishing pigs, but it may not always be economical to include soybean oil in diets fed to growing-finishing pigs.

Dietary fat in diets fed to lactating sows increases milk fat yield and results in heavier pigs being weaned (Tilton et al., 1999; van den Brand et al., 2000). Soybean oil has been shown to be effective in promoting these improvements (Yen et al., 1991), but to our knowledge, there are no studies comparing the effects of soybean oil to the effects obtained from other fat sources.

Full fat Soybeans

Soybeans used in diets fed to pigs are usually defatted prior to usage and only the resultant soybean meal is used. However, full fat soybeans may also be used in diets fed to pigs provided that they have been heat treated prior to feeding. Because of the relatively high oil content in full fat soybeans, the energy concentration of diets usually is improved if full fat soybeans are included. The digestibility of AA in full fat soybeans is greater than in soybean meal (Cervantes-Pahm & Stein, 2008) and the concentration of DE and ME in full fat soybeans is also greater than in soybean meal (Woodworth et al., 2001; Baker & Stein, 2008).

Full fat soybeans are often included in diets fed to nursery pigs. However, diets fed to growing and finishing pigs usually do not contain full fat soybeans because the oil in full fat soybeans may reduce the quality of the belly of the pigs. It has been demonstrated that diets containing full fat soybeans may completely replace soybean meal in diets fed to growing finishing pigs without any negative impact on pig performance (Leszczynski et al., 1992). If pigs are swished to a regular corn-soybean meal based diet 3 weeks prior to slaughter then there is no negative effects on belly quality of feeding full fat soybeans (Leszczynski et al., 1992).

Full fat soybeans may be included in diets fed to sows and can potentially replace all soybean meal in gestation as well as lactating diets.

ISOFLAVONES IN SOYBEAN PRODUCTS

Soybean products contain isoflavones, which is a subgroup of flavonoids, in concentrations between 100 and 5,000 ppm (Wang & Murphy, 1994). The main isoflavones in soybeans are genistein, daidzein, and glycitein. Isoflavones included in diets fed to pigs at the levels supplied by typical corn-soybean meal based diets do not negatively affect growth performance of pigs (Payne et al., 2001), but inclusion of genestein in diets fed to swine in concentrations of 200 to 400 ppm may enhance systemic serum virus elimination in virally challenged pigs (Greiner et al., 2001). To our knowledge, there have been no specific safety assessments of soy isoflavones in diets fed to swine, but it has been demonstrated that inclusion of isoflavones at concentrations that are 5 times greater than what is typically provided by soybean meal does not negatively influence pig growth performance (Payne et al., 2001). The fact that soybean meal is considered the primary protein source for the production of more than 100 million pigs annually in the US also indicates that no negative reproductive or developmental effects are associated with the feeding of soybean protein to pigs and that the levels of isoflavones in soybean meal can be considered safe. In my opinion, the very high nutritional performance requirements of commercial swine production make it a very sensitive indicator of isoflavone toxicity if that was a significant problem. Because the metabolism of isoflavones is similar in pigs and in humans (Gu et al., 2006) it is very likely that humans also tolerate the presence of soy isoflavones in their diets.

CONCLUSIONS

Extruded soybean meal is a highly popular feed ingredient in diets fed to all categories of swine except for newly weaned pigs. The main reason for including soybean meal in diets fed to pigs is to provide AA that are required by the animals. The digestibility to pigs of AA in soybean meal has been measured in numerous experiments and results have shown that the digestibility is relatively high and relatively constant in various sources of soybean meal. In contrast, there are only few reports on the digestibility of P in soybean meal, and the results are somewhat conflicting. However, it is recognized that the digestibility of P in soybean meal can be increased by more than 100% if microbial phytase is added to the diet. There is also a need for more information on the concentration of digestible energy in soybean meal. Soy protein isolate and soy protein concentrate can be used in diets fed to newly weaned pigs Fermented soybean meal and enzymatically prepared soybean meal have recently become available to the industry. These products may potentially replace soy protein concentrate and soy protein isolate in diets fed to weanling pigs.

In conclusion, soybean products are excellent protein sources that may provide all AA for reproducing and growing pigs at all physiological stages. More than 100 million pigs are produced annually in the US and fed diets based on soybean meal. These diets contain relatively large quantities of soy isoflavones. The fact that this feeding practice has not impaired or negatively influenced animal reproductive and developmental performance indicates that there are no negative effects of the soy isoflavones in diets fed to pigs.

Respectfully submitted,

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Table 1. Concentration of energy, P, and AA in soybean products fed to swine [1]

Item	Soybean meal		Full fat	Soy protein	Soy protein
	Non-dehulled	Dehulled	soybeans	concentrate	isolate
Energy, kcal DE/kg	3,490	3,685	4,140	4,100	4,150
Energy, kcal ME/kg	3,180	3,380	3,690	3,500	3,560
Crude protein, %	43.8	47.5	35.2	64.0	85.8
Phosphorus, %	0.65	0.69	0.59	0.81	0.65
Calcium, %	0.32	0.34	0.25	0.35	0.15
Amino acids, %					
Arginine	3.23	3.48	2.60	5.79	6.87
Histidine	1.17	1.28	0.96	1.80	2.25
Isoleucine	1.99	2.16	1.61	3.30	4.25
Leucine	3.42	3.66	2.75	5.30	6.64
Lysine	2.83	3.02	2.22	4.20	5.26
Methionine	0.61	0.67	0.53	0.90	1.01
Cysteine	0.70	0.74	0.55	1.00	1.19
Phenylalanine	2.18	2.39	1.83	3.40	4.34
Tyrosine	1.69	1.82	1.32	2.50	3.10
Threonine	1.73	1.85	1.41	2.80	3.17
Tryptophan	0.61	0.65	0.48	0.90	1.08
Valine	2.06	2.27	1.68	3.40	4.21

[1] All data from NRC (1998).

Table 2. Standardized ileal digestibility of amino acids (%) in soybean products fed to swine [1]

Item	Soybean meal		Full fat soybeans	Soy protein concentrate	Soy protein isolate
	Non-dehulled	Dehulled			
Arginine	93	94	93	99	99
Histidine	90	91	88	97	91
Isoleucine	88	89	84	95	90
Leucine	88	89	86	95	89
Lysine	89	90	86	95	91
Methionine	91	91	85	94	92
Cysteine	84	87	80	94	82
Phenylalanine	88	89	88	97	92
Tyrosine	90	90	87	96	91
Threonine	85	87	83	94	85
Tryptophan	87	90	82	93	88
Valine	86	88	83	94	89

[1] Data for soy protein isolate measured in weanling pigs (Cervantes-Pahm & Stein, 2008). All other data measured in growing-finishing pigs (NRC, 1998).

Table 3. Analyzed nutrient composition of soybean meal, HP 300, and PepSoyGen (%, as-is basis)[1]

Item	Soybean meal	HP 300	PepSoyGen
DM	89.32	91.48	91.33
CP	45.07	54.40	53.74
Ether extract	1.07	1.13	0.80
Crude fiber	2.78	3.75	3.31
Ca	0.26	0.35	0.29
P	0.67	0.74	0.82
Glucose	0	0.49	0.36
Sucrose	7.81	0	0
Fructose	0.63	1.11	0.70
Stachyose	5.17	0.71	0
Raffinose	1.08	0.16	0
Indispensable AA			
Arg	3.06	3.75	3.50
His	1.13	1.35	1.30
Ile	1.89	2.31	2.48
Leu	3.37	3.98	4.09
Lys	2.77	3.06	3.11
Met	0.63	0.71	0.76
Phe	2.23	2.74	2.71

Thr	1.71	2.02	1.98
Trp	0.62	0.69	0.67
Val	1.96	2.40	2.69
Dispensable AA, %			
Ala	1.86	2.25	2.29
Asp	4.80	5.71	5.67
Cys	0.67	0.76	0.77
Glu	7.48	8.75	8.56
Gly	1.77	2.26	2.23
Pro	2.08	2.46	2.45
Ser	1.97	2.35	2.24
Tyr	1.67	2.03	1.97

[1] Data from Cervantes-Pahm, 2008.

Table 4. Standardized ileal digestibility (%) by weanling pigs of crude protein and amino acids in soybean meal, HP 300, and PepSoyGen [1, 2]

Item	Soybean meal	HP 300	PepSoyGen
CP	80.3	92.2	82.2
Indispensable AA			
Arg	90.9	98.2	93.5
His	84.0	88.9	84.4
Ile	82.9	89.8	85.8
Leu	82.0	89.3	85.4
Lys	79.2	88.3	77.2
Met	85.5	92.2	88.3
Phe	84.1	91.9	87.2
Thr	77.4	85.8	78.5
Trp	84.8	87.5	83.5
Val	81.9	89.5	84.3
Dispensable AA			
Ala	77.0	88.7	81.0
Asp	79.5	88.3	81.7
Cys	73.4	85.2	69.7
Glu	81.1	93.7	84.2
Gly	65.0	94.9	74.6
Pro	120.7	149.4	132.5

Ser	82.5	89.4	82.2
Tyr	86.1	92.1	87.7

[1] Data from Cervantes-Pahm.

[2] Data are means of seven observations per treatment.